

Investigating the structure of monomeric and fibrillar $\beta_2\text{m}$ by mass spectrometry

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Introduction.

β_2 -microglobulin ($\beta_2\text{m}$) is a small protein of 11,860 Da, 99 amino acids in length, which readily forms amyloid-like fibrils in acidic conditions *in vitro*. *In vivo* $\beta_2\text{m}$ is found within the plasma at low concentrations. However, in patients suffering from chronic kidney disease the concentration of $\beta_2\text{m}$ can increase up to 60 fold, and this can lead to formation and deposition of amyloid fibrils within collagen rich areas of the body, such as the joints. At this time it is not known how $\beta_2\text{m}$ forms amyloid fibrils *in vivo* at a more neutral pH where the protein is in its native state fold and is known to be stable. NMR and CD indicate that the protein is partially folded at \sim pH 3.6 and is largely unfolded at \sim pH 2.5 where the protein forms amyloid fibrils *in vitro*. Our group is investigating the structure of monomeric and fibrillar $\beta_2\text{m}$ by mass spectrometry.

Charge state distributions of $\beta_2\text{m}$.

Electrospray ionization mass spectrometry (ESI-MS) can be used to identify different conformers of a protein, as unfolded species tend to carry on average more charges. By assigning Gaussian distributions to the charge state distribution (CSD) at different pH values it is possible to identify three different conformations of $\beta_2\text{m}$. The ratios of the three conformers vary depending on the pH with a more expanded conformer of the protein present at very low pH (pH 1.0-2.6), a partially expanded conformer present between pH 2.6 and 5.4, and a compact conformer at pH 5.4-6.0. These represent the acid unfolded, partially folded and native states of the protein, respectively. Two mutants that are known by other techniques to be globally destabilised were compared with wild-type $\beta_2\text{m}$ using the charge state distributions one of which is shown in Fig. 1. The CSDs of all three proteins can be fitted to the same Gaussian distributions as the wild-type protein, although the pH at which different species become populated during pH denaturation was protein-specific. The mutants populate proportionally higher concentrations of partially and acid unfolded conformers at higher pH than the wild-type protein. The data provide a unique opportunity to delineate and quantify species co-populated in solution, one or more of which may be important in amyloid formation.

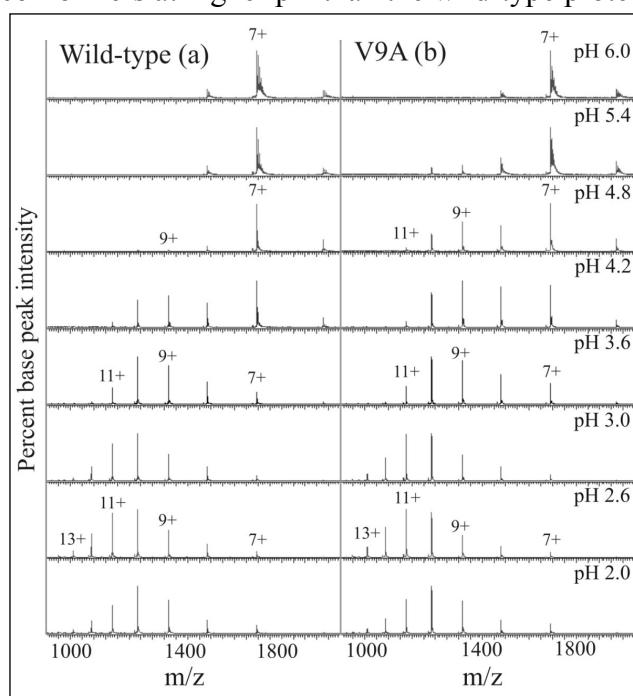


Fig. 1: Mass spectra taken at different pH values showing the shift in the CSD from a compact distribution centred on the 7 charge state at pH 6 to multiple CSD at lower pH. The transition between different protein conformers occurs at different pH for the two proteins. a) Wild-type $\beta_2\text{m}$, b) the mutant V9A $\beta_2\text{m}$ ¹.

Monitoring β_2 m assembly into amyloid fibrils using mass spectrometry.

Although ESI-MS provides data with high mass accuracy, the technique is not usually used to quantitate protein concentrations, since it is not possible to relate the observed peak heights for a specific protein to its concentration in solution. However, by using an internal standard of known concentration, it is possible to create a calibration across a concentration range for a protein of interest, hence making the method semi-quantitative. In this study we have used a constant concentration of bradykinin to follow the concentration of monomeric β_2 m under *in vitro* amyloid forming conditions at pH 3.6. This reveals an exponential decrease in monomer concentration over an 8 hour period until there is less than 20% of the original concentration remaining (Fig. 2a). It is possible to follow the appearance of β_2 m oligomers as the monomer concentration decreases. The molecular weight of the oligomers detected suggests that the process by which amyloid forms under these conditions is by monomer addition, as a series of oligomers from dimer to dodecamer are detected (Fig. 2b). The population of these oligomers decreases at long times of assembly, as higher order oligomers and fibrils are formed. The data provide an exciting framework with which to determine the mechanism of amyloid formation under different conditions and to determine the mechanism of action of potential inhibitors of amyloidosis.

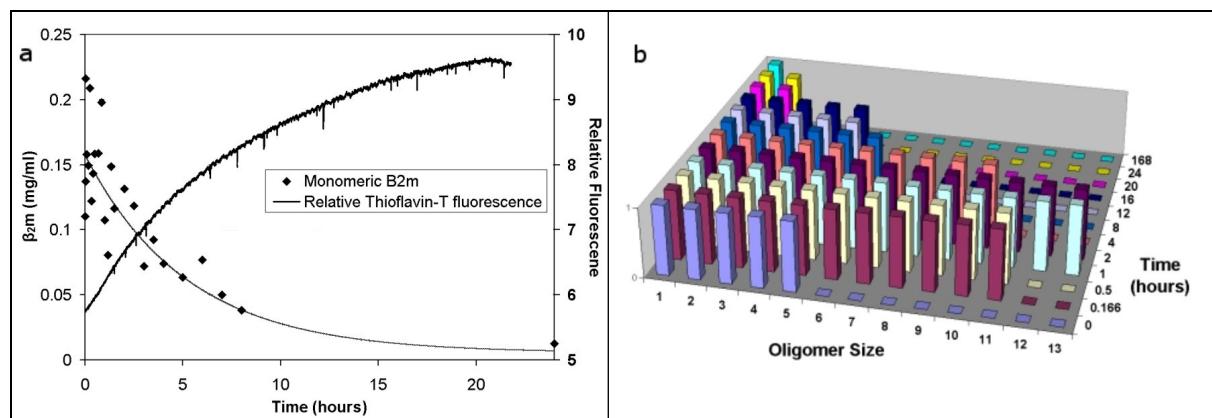


Fig. 2: a) A time course following the concentration of monomeric β_2 m determined by ESI-MS at pH 3.6, 150mM ammonium formate, as amyloid forms. Amyloid fibril formation was also monitored using Thioflavin-T fluorescence. b) A time course following the presence or absence of oligomers of β_2 m at pH 3.6, 150mM ammonium formate at different time points during amyloid assembly.

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Collaborators.

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Publications.

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